Rabies Encephalitis in Humans: Pathology, Pathogenesis and Pathophysiology

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INTRODUCTION

Rabies. The word itself immediately commands attention. A gentle and loving pet suddenly ferocious and threatening. An irrational and aggressive wild (truly wild) animal, stripped of its usual fear of humans, emerging from the woods. A nocturnal attack by a bat. Images of painful and interminable intrathecal postexposure treatments (now obsolete) and of delirium and rage in a dying human victim. There is perhaps no other disease known to man whose public recognition and fear stand in such stark contrast to its actual modern American incidence. Indeed, the very rarity of the disease adds to its exotic and nightmarish image. The physician no less than the layman pauses at the mention of this diagnosis. The unique mode of transmission, the virtually exclusive neurotropism shown by the agent and the complete hopelessness of established disease set rabies far apart from other endemic zoonoses rarely transmitted to man.

HISTORY

Rabies' prominence in human consciousness is not new (1, 2). Written accounts of rabid behavior in dogs date back more than four millennia to the Mesopotamian Code and the Babylonian Code of Eshnunna (ca. 2300 B.C.). The great Roman physician Celsius associated hydrophobia with dog bites around 100 A.D., and the irreversibility of symptomatic disease was appreciated by Maimonides (1198 A.D.).

Early attempts to diagnose rabies in animals involved examining stomach contents for sticks and stones, as evidence of deranged behavior. Pasteur, in 1885, demonstrated the transmissibility of rabies by inoculation using infected brain tissue. Babes described the microglial nodules which bear his name in 1892 (3), and the diagnostic cytoplasmic intraneuronal inclusions of rabies were described (but mistakenly identified as protozoan organisms) by Adelchi Negri in 1903 (4). Indeed, Negri bodies remain a specific (albeit not particularly sensitive) diagnostic lesion of rabies infection. Modern techniques of rabies diagnosis date from 1958, when Goldwasser and Kissling developed a direct immunofluorescent antibody technique for demonstration of rabies antigen in tissue (5).

Rabies in the United States dates from the 1700s, when rabid foxes and dogs were noted in the English colonies. Domestic dogs and cats have historically been a major source of rabies, but the introduction of animal vaccination programs in the 1940s markedly decreased the incidence of rabies in these animals in the U.S. By 1960 most reported U.S. cases of animal rabies were in wild animals and today well over 80% of all reported cases are in wild animals. Indeed, today most reported cases of rabies in dogs and cats occur in farm or feral animals in areas endemic for skunk rabies. Concurrent with this success in the control of rabies in domestic animals, reported cases of rabies in humans have declined from levels of 50–60 cases/year prior to the 1940s to about one case/year today (6). However, almost 20,000 people each year are still treated with postexposure rabies vaccination protocols. Indeed, with the availability and success of postexposure prophylaxis, the majority of human cases reported in the U.S. over the last decade actually occurred in patients with no history of exposure (7, 8). Worldwide, in contrast, rabies remains widespread among domestic animals, and tens of thousands of people die of rabies each year.

EPIDEMILOGY

Endemic animal rabies occurs on every continent except Australia and Antarctica, and in every American state except Hawaii (9). Rabies virus is capable of infecting any warm-blooded animal, but today in the U.S. most reported cases of animal rabies involve skunks, raccoons, bats and foxes (10). These animal species often harbor genetically distinct rabies strains which can be distinguished antigenically using monoclonal antibodies (11). Skunk rabies occurs in a broad central band from Wisconsin to Montana and south to Texas; in a second band stretching from Tennessee and Kentucky to New Jersey, and in northern California. Raccoon rabies has long been endemic in southern Atlantic Coast states (South Carolina, Georgia, Florida) and has spread to middle Atlantic states (Virginia to New Jersey) following the 1978 introduction of rabies-carrying raccoons into the area by West Virginia hunting clubs. Fox rabies occurs in Alaska.
and, in small numbers, in East Coast states. Bat rabies is found throughout the 48 contiguous states. Rabies may also occur in rodents, especially woodchucks. However, experimentally infected rodents usually do not shed rabies virus in their saliva, and there is no documented human case of rabies resulting from a rodent bite. Direct human-to-human transmission is theoretically possible but is exceedingly rare if, indeed, it occurs at all (12, 13).

THE RABIES VIRION

Rabies virus is a member of the rhabdovirus family (rabdo [Greek] = rod-shaped) and is one of two viruses in this family (along with vesicular stomatitis virus) capable of infecting humans. Rabies virions are rod- or bullet-shaped particles measuring 75 × 180 nm (Fig. 1) and containing a ribonucleoprotein nucleocapsid core surrounded by a tight, lipoprotein-containing envelope (14).

The rabies virus envelope membrane contains two viral proteins which make up 50% of the membrane dry weight. An externally oriented, integral membrane glycoprotein (G protein) is the basic unit of the ultrastructurally demonstrable multimeric surface projections. This protein, the only externally exposed protein in the intact rabies virion, is the major antigenic determinant and the primary target for neutralizing antibodies and for rabies-immune T-helper and cytotoxic T cells. This G protein, essential for pathogenicity, shows little variation between rabies strains, and rabies immunity thus transcends these strains. A second envelope protein, the matrix protein, lines the inner surface of the virion membrane and anchors the nucleocapsid to this membrane. This protein shows considerable interstrain variability and is the major antigenic determinant currently used in typing of rabies virus strains for epidemiological analysis. The rabies nucleocapsid, the infectious component of the virus, contains an RNA genome and an RNA-dependent RNA polymerase complex composed of three proteins: a large (L) protein, a nonstructural (NS) protein (also called the P protein) and a core nucleocapsid (N) protein. The genome is an unsegmented, single-stranded, negative-sense RNA which requires its own viral RNA polymerase for transcription (i.e. it cannot function as an mRNA). The genome is tightly surrounded by the N protein.

SYSTEMIC PATHOPHYSIOLOGY

Rabies virions, introduced into deep soft tissue by an animal bite, initially infect muscle, possibly through an affinity for nicotinic acetylcholine receptors (15–17). The virus replicates locally in muscle fibers (18, 19) before achieving access to the nervous system through either neuromuscular spindles (18) or muscle motor end plates (15). Alternatively, rabies virions introduced into superficial soft tissues may directly infect adjacent sensory nerve endings. Aerosol transmission of rabies, although rare, has been documented in spelunkers visiting bat-infested, humid caves (20) and in laboratory workers (21, 22). In these cases the rabies virions directly infect exposed neuroepithelial cells of the olfactory end organ in the nose (23). Rare cases of rabies acquired from infected corneal transplants have also been reported (24).

Rabies virus reaches the central nervous system early in the disease and returns to the periphery late in the disease by intra-axonal transport. Both anterograde and retrograde transport occur (25) via a colchicine-sensitive mechanism (26, 27), probably involving both sensory and motor pathways (28). Experimentally determined viral axonal transport rates are 50–100 mm/day in human tissue (29). Rabies viremia has never been observed at any stage of the disease, and this absence of detectable extracellular virus explains in part the absence of detectable immune response until late in the disease. Furthermore, intact viral particles are not seen during the early phase of centripetal movement, even during trans-synaptic movement from one neuron to the next (30). This trans-synaptic movement is of some interest. This appears to be a passive process, rather than receptor-mediated, and is decidedly not restricted to cholinergic neurons (30).

The viral nucleocapsids may take advantage of ongoing secretory (neurotransmission) and membrane reorganization activity, or perhaps take advantage of spinules, small finger-like processes of postsynaptic cells invaginating into, and pinching off inside of, presynaptic terminals (30). This initial incubation period of centripetal rabies movement toward the central nervous system is asymptomatic and classically thought to require 10–90 days, depending in part on the anatomic distance between the inoculation site and the brain. Recent reports have suggested that much longer incubation periods (years) are possible (8). Muscle may be the site of viral sequestration.
in cases with long incubation periods (19), but this remains unproven.

Clinical illness begins upon arrival of the virus in the central nervous system, with nonspecific flu-like symptoms. Pain or paresthesia at the original exposure site (seen in 50% of patients) may be the first rabies-specific symptom. During this stage, neurological involvement may be suggested by symptoms such as apprehension, anxiety, agitation, irritability, nervousness, insomnia or depression. This prodromal period generally lasts 2–10 days, during which there is still no detectable antibody or inflammatory response to the infection. The pattern of viral spread within the central nervous system during this period is largely defined by the original site of inoculation and the centrifugal pathways subsequently followed by the virus. There appears to be an initial proclivity toward infection of the diencephalon, hippocampus and brainstem, but the virus will eventually spread to all parts of the central nervous system, both via intercellular spread to adjacent neurons (19, 31) and via intra-axonal spread along neural pathways (32, 33). The early localization of the virus in the limbic system, with cortical sparing (34), correlates clinically with behavioral and emotional changes in an alert and cognitively intact patient. In animals, this stage promotes transmission of the virus through aggressive attacks and bites of other animals. Also in animals, involvement of the hypothalamus and adenohipophysis at this point, with consequent loss of somatotrophic activity, correlates with progressive wasting, thymic atrophy and generalized lymphoid depletion (35).

Objective neurological involvement heralds the acute neurological phase of the illness. Intact rabies virus particles are synthesized and direct neuron-to-neuron spread occurs, with widespread dissemination of the virus throughout the central nervous system in addition to continuing trans-synaptic spread along neural pathways (30). It is also at this point that serum antibodies to rabies virus first appear, to be followed within a week by cerebrospinal fluid antibodies to rabies. This latter development distinguishes active rabies infection from the antibody response generated by rabies vaccination, which does not result in detectable cerebrospinal fluid antibodies. Neurological signs may be “soft,” such as hyperactivity, disorientation, bizarre behavior and hallucinations, as well as “hard,” such as muscle fasciculations, seizures, nuchal rigidity and even paralysis. The latter may be ascending, mimicking Guillain-Barré polyneuritis. The hyperactivity and bizarre behavior is typically episodic, lasting a few minutes, and alternating with periods of calm during which the patient is often cooperative and oriented. The classic hydrophobia, or pharyngeal and laryngeal spasm when attempting to drink, is only seen in 30–50% of patients, although most patients manifest some degree of difficulty in swallowing. Some degree of fever is almost always present. It is at this point that patients usually present to a hospital, and the various combinations of these neurological symptoms may suggest a variety of diagnoses, including epilepsy, encephalitis, meningitis, tetanus, drug toxicity, hysteria and inflammatory polynepath.

Paralytic human rabies, a clinical variant characterized by seizures and constant, high fever, is a less common alternative to encephalitic rabies (36, 37) and one which corresponds with “dumb” (as opposed to “furious”) rabies in animals. This variant is characterized pathologically by perivascular inflammatory infiltrates and microglial proliferation with neuronal destruction primarily restricted to the brainstem and spinal cord. Inclusion bodies are sparse (36). Paralytic rabies appears to correlate with absence of significant immune response to the infection. These patients, unlike those with encephalitic rabies, show diminished numbers of peripheral blood B cells (38). They furthermore lack the cellular reactivity against rabies antigen demonstrable by the lymphocyte proliferation test, lack the elevated serum soluble interleukin-2 receptor (an indicator of lymphocyte proliferation), and lack the elevated serum interleukin-6 levels which are features of encephalitic rabies patients. Serum T cell levels are comparable in the two types of rabies, with natural killer cell numbers depressed in all patients (37–39). Experimental rabies infection in immunosuppressed mice suggests that an immune reaction mounted at late stages in the illness may exacerbate rather than alleviate clinical symptoms (40).

Replication of the rabies virus in the central nervous system is followed by centrifugal movement of rabies virus, again along peripheral nerve axons, back to peripheral tissues. At this late stage, intact viral particles, complete with envelopes, may be found in the peripheral nerve axons, in contrast to the exclusive presence of bare nucleocapsids during the centrifugal phase of viral migration. These viral particles are often in vesicles, surrounded by endoplasmic reticulum membrane (30), which suggests that they originate from intracellular budding into the endoplasmic reticulum rather than from extracellular budding through the plasma membrane. Peripherally, there is a particular but unexplained tropism for salivary glands and lacrimal glands, and infectious rabies particles appear in saliva and tears, and possibly in other tracheobronchial secretions. Trans-synaptic infection of the heart may cause a rabies myocarditis (41, 42).

Following 2–7 days of acute neurological symptoms, patients succumb to apathy and then coma, corresponding with widespread cortical rabies infection. Death is usually attributable to failure of basic central vegetative functions but may be due to concomitant rabies myocarditis. This generally occurs within 1–2 weeks in unsupported patients but may be delayed somewhat by intensive supportive care. Avoidance of such unnecessary
Figs. 2-4.  Fig. 2. Well-defined, round eosinophilic inclusions (Negri bodies) in a Purkinje cell (a) and in a pigmented substantia nigra neuron (b). Human rabies encephalitis (7). These inclusions may be sparse or even completely absent in rabies encephalitis, particularly in those cases with short clinical duration. H&E, ×850. Fig. 3. Rabies viral inclusions (four) in a human Purkinje cell (7) stained with basic fuchsins and toluidine blue. Light microscopy of semi-thin ("thick") section of epoxy-embedded material. The inclusions are fuchsinophilic. ×850. Fig. 4. Irregular eosinophilic rabies virus inclusions ("lyssa bodies") in a human Purkinje cell. H&E, ×850.

Figs. 7-10.  Fig. 7. Microglial nodule (Babes nodule) in the medulla of a human rabies patient (7). Although these lesions were originally described in cases of rabies, histologically identical lesions are found in many viral encephalitides as well as in other conditions. H&E, ×340. Fig. 8. Immunofluorescent demonstration of rabies viral antigen (green) in an impression of fresh brain tissue from a cat. In contrast to the frequent scarcity of viral inclusions in rabies-infected brain, immunoreactive rabies antigen is generally easily demonstrated by this technique. Polyclonal FITC-labeled anti-rabies antibody. Courtesy of Dr. Deborah J.
RABIES ENCEPHALITIS IN HUMANS

CELLULAR PATHOPHYSIOLOGY

Rabies virus replicates only in the cytoplasm of eukaryotic host cells. Entry is achieved by adsorption of the virus through an attachment between the viral envelope G protein and host cell membrane phospholipids. Both viral sialic acid residues (43) and host cell glycolipids (44, 45) appear to be involved in this process. Indeed, the natural neuronal richness in glycolipids may explain in part rabies viral tropism toward neurons. Adsorption is followed by penetration of the cell, either by fusion of the viral and host cell membranes or by endocytosis of the adsorbed virus into coated pits and then coated vesicles (44). In the latter event, uncoating of the nucleocapsid is accomplished by fusion between viral and endocytic vesicle membranes, facilitated by the acidic interior of the vesicle (44). The viral nucleocapsid genome, now free in the cytoplasm, is transcribed by viral RNA transcriptase, which self-assembles following association of the N protein with the viral RNA. Five viral monocistronic mRNAs are produced, capped and polyadenylated, and a leader RNA is produced without capping or polyadenylation. Immediate and continuing translation of the five viral mRNA ensues; four viral proteins (the L, NS, N and matrix proteins) are produced directly while the fifth, the G protein, is produced following translation by rough endoplasmic reticulum-associated polyribosomes and post-translational glycosylation of the protein product. This glycosylated G protein migrates with cytoplasmic vesicles to the cell surface where fusion between the vesicles and the surface membrane inserts G protein into the host cell membrane. Viral genome replication is accomplished by the viral transcriptase in two steps: first, synthesis of a complete complementary positive-sense RNA molecule and, second, use of this positive-sense template to synthesize complete negative-strand viral RNA molecules. Binding of these progeny RNA molecules to N, L and NS proteins produces newly assembled nucleoprotein cores. Matrix protein then binds these nucleocapsid cores to the G protein-containing host cell membrane. Envelopment of the nucleocapsid by host cell membrane and consequent budding of the infectious progeny virus from the cell completes the process. Budding into G protein-containing endoplasmic reticulum cisterns also occurs (19) and is a characteristic ultrastructural feature of the Negri body (7). During the early stage of centripetal movement along peripheral nerve axons toward the central nervous system, intact rabies virus particles are not demonstrable either within axons or traversing synaptic spaces. Apparently, naked nucleocapsid particles accomplish this spread, and intact, enveloped virions are produced only following infection of the brain. Rabies virus replication is apparently restricted to neurons and muscle, although rabies antigens can also be demonstrated in neuroglial cells (46). Rabies virus does not cause neuronal cell lysis, and the cause of neuronal death in rabies is not entirely clear. Recently, rabies-induced synthesis of inducible nitric oxide synthase, with subsequently elevated levels of nitric oxide in the central nervous system, has been suggested as a pathogenetic step in neuronal cell damage in rabies infections (47).

DIAGNOSIS

Pathological diagnosis of rabies encephalitis rests on (1) demonstration of anti-rabies antibodies, either as rising serum titers or as cerebrospinal fluid titers, (2) histological or immunohistochemical demonstration of the virus in tissue, or (3) viral recovery, either by animal inoculation or by culture in vitro. No currently available technique is capable of establishing a diagnosis of rabies prior to the onset of clinical symptoms.

Serum anti-rabies antibodies appear about the time of onset of neurological symptoms, and detectable cerebrospinal fluid antibodies follow within a week. Enzyme immunoassays, immunofluorescence assays and radioimmunoassays are available for detection of these antibodies; all of these tests measure directly the interaction of (patient) antibodies with rabies antigens. In addition, functional antibody tests, such as complement fixation, immunoadherence hemagglutination, complement-mediated cell lysis, passive hemagglutination, mixed hemadsorption and counter immunoelectrophoresis are available. These latter tests are quite sensitive to IgM and are thus particularly useful during the early stages of antibody response to infection.

Histological diagnosis of rabies was for many years dependent on the postmortem demonstration of cytoplasmic viral inclusions in infected neurons (48–50) (Fig.

Briggs, Kansas State University. Fig. 9, a, b: Immunofluorescent demonstration of rabies viral antigen (green) in small parafollicular nerve fibers in a skin biopsy from a cat. Polyvalent FITC-labeled anti-rabies antibody. Courtesy of Dr. Deborah J. Briggs, Kansas State University. Dr. Dennis Howard (deceased) performed the photography. Fig. 10. Immunoperoxidase demonstration of rabies viral antigen in rat cerebellum using a peroxidase-antiperoxidase technique for formalin-fixed, paraffin-embedded material. There is intense immunoreaction in the Purkinje cell soma and dendrites (arrow). This particular specimen was embedded in paraffin three years prior to immunoreaction. This new technique is specific for rabies and allows evaluation of archival material but is not as sensitive as older immunofluorescence techniques using unfixed tissue. Polyvalent anti-rabies nucleocapsid antibody. Courtesy of Dr. Manuel J. Torres-Anjel, University of Missouri.

2). These inclusions (which may be absent, especially in cases with short clinical courses) are found throughout the central nervous system. They are most abundant in large neurons of the brainstem, hippocampus and cerebellum (Purkinje cells). In humans, Purkinje cells are the most promising site (48). This is in contrast to animals, in which hippocampal inclusions are more frequent. Classic Negri bodies are round or oval, discrete, sharply demarcated eosinophilic bodies with fine basophilic stippling. The basophilic stipples are particularly important in diagnosing infections in cats, rodents, monkeys and cattle, as nonspecific eosinophilic inclusions in these hosts may be mistaken for Negri bodies. In humans, the basophilic stippling is less common and less important. Negri bodies stain with fuchsin (Fig. 3), and this is the basis of Seller’s stain (fuchsin and methylene blue) for Negri bodies. In addition to the round, discrete Negri bodies, there are other inclusions that are irregular and less sharply demarcated from the surrounding neuronal cytoplasm. Such “lyssa bodies” (Fig. 4) are less specific, but usually more common, than Negri bodies. Ultrastructurally, both Negri bodies and lyssa bodies show a central filamentous or granular core surrounded by viral nucleocapsids budding into dilated cisternae of endoplasmic reticulum (7, 51) (Fig. 5), and this ultrastructural appearance is diagnostic. Some degree of inflammation is characteristic, but not universal. Perivascular cuffs of lymphocytes and plasma cells (Fig. 6), microglial nodules (Fig. 7) and neurono-

Fig. 5. Electron microscopy of a rabies viral inclusion in a Purkinje cell from a case of human rabies encephalitis (7). A central core (C) of filamentous material is surrounded by a rim of electron-dense viral particles, some of which are budding into dilated cisterns of endoplasmic reticulum (*). These ultrastructural features, which are diagnostic of rabies infection, are common to both the (histologically) diagnostic Negri bodies and the (histologically) more equivocal lyssa bodies. $\times 21,400$.

Fig. 6. Perivascular inflammation in the medulla of a human rabies patient (7). H&E, $\times 300$. 

phagia are usually found in the brainstem and spinal cord and appear somewhat earlier in the disease than do the diagnostic neuronal inclusions. An unusual histopathological feature of rabies encephalitis is the frequent spatial dissociation between the inflammation and the inclusion-bearing neurons. Considerable variability in inflammatory response (48) may be due in part to differences between infecting strains of rabies virus (52).

Immunohistochemistry, particularly fluorescence immunohistochemistry, has largely supplanted classical histological examination. Usually this is performed using a polyclonal anti-nucleocapsid antibody or an IgG against rabies intracytoplasmic nucleocapsid (N) antigen (53). Rabies viral antigen is frequently demonstrable in cases in which Negri bodies are absent and is always present in many more cells than those containing inclusions (46, 54). Furthermore, rabies antigen may be demonstrated in tissues other than central nervous system, including corneal impressions (55), nasal mucosa, and cutaneous nerves (56), thus enabling premortem immunohistochemical diagnosis. All of these techniques work best using fresh tissue and fluorescent-labeled antibodies. The application of this technique to either smears (Fig. 8) or frozen sections of autopsy tissue is straightforward, and the sensitivity approaches 100% when compared with viral isolation by intracerebral mouse inoculation. Indeed, fluorescence immunohistochemistry will occasionally demonstrate rabies antigen in cases in which viral isolation fails, a phenomenon possibly explained by neutralization of the inoculated virus by high patient antibody titers. Thorough immunohistochemical examination should include sections from brainstem, cerebellum and hippocampus from both the right and left sides of the brain.

Premortem immunohistological diagnosis may be made using corneal impressions, nuchal skin biopsies, or biopsies of oral or nasal mucosa. These techniques are dependent upon centrifugal transport of rabies virus to peripheral nerves late in the disease, and will thus be negative in the early stages of clinical illness. Brain biopsy is, of course, potentially diagnostic as well but is rarely employed. Corneal impressions are prepared by vigorously pressing a clean microscope slide against the eyeball (55). The pressed cells are then fixed, stained and read according to usual immunofluorescent techniques. Nuchal skin biopsies depend upon the demonstration of rabies antigen in small cutaneous nerves adjacent to hair follicles (56) (Fig. 9), and an adequate biopsy should contain at least 10 such follicles and be at least 3–6 mm in diameter. The biopsy should be sent unfixed and without added fluids for prompt frozen sectioning or frozen on dry ice if shipping is necessary. Prompt, rapid shipping and avoidance of desiccation are essential. The specificity of all of these techniques is essentially 100%, but the sensitivity varies. For skin biopsies, positive results are reported for 68 (97%) of 70 clinically ill rabid animals (57). For human patients, positive results are reported for 3 (60%) of 5 single nuchal biopsies obtained after onset of clinical encephalitis (56). The corneal impression technique gave positive results for 13 (31%) of 42 human rabies patients in one study (55). Reported sensitivities for oral and nasal biopsies are somewhat lower.

Recently, immunohistochemical techniques have been developed for fixed, paraffin-embedded tissues (58–60) (Fig. 10). While a positive result with such an analysis is diagnostic, these techniques lack the sensitivity of procedures employing fresh tissue and are thus useful primarily for retrospective analysis of cases for which no unfixed tissue is available. Using a peroxidase-antiperoxidase technique, Palmer et al (58) demonstrated rabies antigens in 26 (76%) of 34 animals with known rabies infection. Positive results have been obtained using tissues kept in formalin or embedded in paraffin for many years prior to analysis with either peroxidase-antiperoxidase (59) or avidin-biotin techniques (60).

Viral recovery may be accomplished by intracerebral inoculation of patient brain tissue into mice or by viral culture in vitro using mouse neuroblastoma cells (61). The former approach involves following the inoculated animal for 7–30 days for behavioral evidence of rabies followed by immunohistochemical analysis of cerebral tissue. The latter technique is the most sensitive technique available for fresh tissue obtained premortem or immediately postmortem. However, badly decayed tissue or, occasionally, tissue from patients with high antibody titers against rabies may show positive immunofluorescent reaction for rabies antigen and yet fail to yield virus in culture.

PREVENTION AND TREATMENT

Clinical rabies infection is virtually always fatal in non-immunized humans. The few reported survivors of clinical, symptomatic rabies all had some degree of previous immunity or received some degree of postexposure prophylaxis prior to clinical illness (22, 62). Successful prophylactic treatment of rabies exposure depends upon preventing clinical illness through thorough wound cleansing, postexposure vaccination and administration of human rabies-hyperimmune globulin (63, 64). Such treatment, administered properly and in a timely fashion, virtually guarantees protection from clinical rabies. Treatment is not successful, however, after the onset of clinical illness and, indeed, might even exacerbate rather than alleviate symptoms at this point (37). Postexposure prophylaxis is possible because of the long latent period manifested by this virus, during which the virus travels centripetally to the central nervous system via peripheral nerve axons, usually after peripheral viral replication in infected muscle tissue. Postexposure vaccination is designed to stimulate an endogenous immune response prior to arrival of
the virus in the central nervous system. Administration of human rabies immune globulin is designed to slow peripheral viral replication and axonal transport, thus allowing additional time for successful vaccination response.

Pre-exposure vaccination is also available and is recommended for animal quarantine personnel, veterinarians and their technical staff, workers in rabies laboratories, all contacts of human rabies patients, and individuals working at special risk in enzootic areas (63). A more controversial application is the routine prophylactic vaccination of travelers to foreign countries with high incidences of rabies (65).

Successful vaccination depends upon an antibody response to the rabies surface glycoprotein (G protein), the only rabies protein on the surface of the intact virion. Modern human rabies vaccines, available since 1979, consist of inactivated whole rabies virus grown in human diploid cell (fibroblast) lines. A substantial antibody response usually results from 4–6 intramuscular or intradermal doses of this vaccine (66). Reports of serious anaphylactic, neuroparalytic or encephalitic complications from this vaccine are limited to three cases of self-limited Guillain-Barré-like illness (63). Older methods for preparing rabies viral vaccines, still in use in parts of Asia, Africa and South America, involve growing viruses in duck embryos (a technique developed in the 1950s) or using infected brain tissue from sheep, goats or mice (a technique first used by Pasteur in 1885). The duck embryo vaccine is responsible for local allergic reactions (very common), systemic allergic reactions (about a third of patients) and infrequent neuroparalytic and anaphylactic reactions. This vaccine also requires many more administrations (16–25) to produce satisfactory immunity (67). The nerve tissue vaccines may induce postvaccination allergic encephalitis (0.05–0.5% incidence [63]). They also show somewhat lower efficacy (5–50%) in preventing rabies in exposed humans. An experimental live attenuated virus vaccine, consisting of vaccinia virus expressing rabies surface glycoprotein, has been successfully employed to immunize animals via oral administration. This technique may find application in vaccination of wild animal species that serve as rabies reservoirs (68).

Documentation of seroconversion following rabies vaccination is usually not necessary except in immunosuppressed patients. If documentation is necessary, this should be done using a test specific for anti-G protein IgG, as these are the antibodies conferring rabies immunity. Such virus neutralization assays employ intact live rabies virus and animal inoculation, and are thus available only in specialized laboratories.

Criteria for administration of postexposure prophylaxis have been published by the Centers for Disease Control (63). Bites from available and healthy dogs and cats do not warrant prophylaxis unless the animal shows clinical signs of rabies within 10 days. Bites from known or suspected rabid dogs or cats, or from wild carnivores (particularly skunks, raccoons, bats, foxes and woodchucks) warrant immediate vaccination. There is no documented case of human rabies resulting from bites by rodents, and vaccination is almost never recommended in such situations except for woodchucks. Other situations (bites by dogs not available for examination, bites by domestic livestock, etc.) are to be considered on an individual basis, based in part on the local incidence of rabies in the offending species. The recommendation for a 10-day observation of dogs and cats is based on the presumption that animals incubating rabies will manifest symptoms within this time period. There is, however, considerable variability in the temporal relationship between appearance of the virus in saliva and the onset of clinical illness in dogs (69). Furthermore, there are reports of dogs that carry and secrete rabies virus for months or even years without developing clinical rabies (70–73). The public health policy implications of these latter observations are controversial (74, 75).

GUIDELINES FOR HANDLING OF POTENTIAL CASES

A clinical (i.e. premortem) diagnosis or suspicion of rabies should prompt use of face masks, gowns and gloves by all personnel having contact with the patient. Saliva, cerebrospinal fluid and tissues should be handled and disposed of with proper infectious disease precautions. In addition, all known or suspected human contacts (pre-mortem or postmortem) of a rabies patient should receive postexposure prophylaxis (12).

Suspected or known cases of rabies should be approached at postmortem examination using accepted infectious disease precautions. Rabies virus does not produce a viremia, even late in the disease, and exposure to blood, urine or feces is not a danger. Infectious rabies virions are present in neural tissue (primarily brain and spinal cord, but also adrenal glands and peripheral nerves) as well as saliva, tears, and possibly other oropharyngeal secretions. Presence of rabies virus in visceral tissues other than muscle and salivary glands has not been demonstrated, although rabies antigens have been reported in various other tissues. The extent of peripheral nerve involvement increases with length of clinical illness (and consequent opportunity for centrifugal viral spread), and virtually any innervated tissue must be considered potentially infectious. The rabies virus is rapidly killed by exposure to ultraviolet radiation, sunlight, heat (50°C, 1 hour), solvents (ether, 0.1% sodium deoxycholate) and trypsin. The virus is quite sensitive to desiccation, and dried saliva is not considered infectious. The virus is also inactivated by CO2, and samples shipped on dry ice for viral culture must be tightly sealed. In contrast, rabies virus may survive weeks or months when stored at 4°C.
a source of concern in examining frozen animal carcasses in northern latitudes.

Unfixed, wet brain tissue (fresh, delivered within 48 hours, or frozen in 50% glycerol) should be sent for immunofluorescent examination, generally available in local state health departments. The hippocampus is often preferred by these laboratories, but other areas may be used if necessary. As mentioned above, thorough immunohistochemical evaluation should include sections from brainstem, cerebellum and hippocampus from both the right and left sides of the brain. If immunofluorescent examination is positive, this same tissue may be used for attempts at viral culture and isolation. Pre- or postmortem serum, if available, should be saved for determination of antibody titers. Histological and ultrastructural sampling (less critical for diagnosis) may be guided by the known frequency of histological findings in various brain areas (48), by the interests of the pathologist and, of course, by the requirements of differential diagnosis.

CONCLUDING REMARKS

The postmortem diagnosis of rabies in an unsuspicious case can be difficult. The diagnostic rabies inclusions are sparse or absent in a significant number of cases, and the diagnosis may thus be missed on histological examination. Rabies antigen is easily demonstrated by fluorescent immunohistochemical techniques, but these require unfixed tissue and a specific suspicion of rabies. Immunohistochemical techniques are now available for fixed, paraffin-embedded tissue, but these lack the sensitivity of the fluorescent techniques using unfixed tissue.

Human rabies encephalitis is now a rare disease in the U.S. but is much more common in some parts of the world. Furthermore, significant populations of wild rabid animals persist in the U.S., making rabies a continuing threat. Most American cases now occur in patients without a history of exposure, in part because patients with known exposure receive adequate postexposure prophylaxis and do not develop rabies. The rarity of the disease combined with the now-frequent lack of exposure history suggests that such patients may not receive a definitive, or even provisional, diagnosis of rabies prior to autopsy.

REFERENCES